P[redacted]AU03/01113

Patent Office
Canberra

I, SMILJA DRAGOSAVLJEVIC, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002951082 for a patent by THE CORPORATION OF THE TRUSTEES OF THE ORDER OF THE SISTERS OF MERCY IN QUEENSLAND as filed on 30 August 2002.

WITNESS my hand this
Fifth day of September 2003

S. Dragosavljevic

SMILJA DRAGOSAVLJEVIC
TEAM LEADER EXAMINATION
SUPPORT AND SALES

Regulation 3.2

The Corporation of the Order of the Sisters of Mercy in Queensland

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Therapeutic cellular agents"

The invention is described in the following statement:

THERAPEUTIC CELLULAR AGENTS

FIELD OF THE INVENTION

The present invention relates to a method for inducing development of blood dendritic cells from $CD34^+$ precursor cells. More particularly, the present invention provides a protocol for the development of blood dendritic cells from *inter alia* cord blood, bone marrow or peripheral blood $CD34^+$ stem cells. The blood dendritic cells of the present invention are useful as therapeutic cellular agents such as in the development of vaccines and in modulating immunological responsiveness.

BACKGROUND OF THE INVENTION

Bibliographic details of references provided in the subject specification are listed at the end of the specification.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Dendritic cells (DC) are potent cellular activators of primary immune responses (Hart, *Blood 90:* 3245-3287, 1997). Immature myeloid DC in non-lymphoid organs react to endocytose and process antigens and migrate *via* blood and lymph to T cell areas of lymphoid organs. Here, the mature cells present foreign peptide complexed to MHC Class I and II to T cells and deliver unique signals for T-cell activation (immuno-stimulation). They also stimulate B lymphocytes and NK cells. DC undergo differentiation /activation during this process, lose their antigen-capturing capacity and become mature, immuno-stimulatory DC that trigger naïve T-cells recirculating through the lymphoid organs. The lymphoid DC subset may have a different migration pathway and although capable of stimulating allogeneic and autologous T-lymphocytes they have been suggested to have a regulatory function (Grouard *et al., J. Exp. Med.* 185: 1101-1111, 1997). As part of the

differentiation/activation process, DCs up-regulate certain relatively selectively-expressed cell surface molecules such as the CMRF-44 and CD83 antigens. DC in the thymus and blood that do not have an activated co-stimulating phenotype probably contribute to central and peripheral tolerance.

Blood dendritic cells (BDC) are released from bone marrow into the peripheral blood before homing to the tissues as surveillance DC for the immune system. The two major subsets of BDC are myeloid DC ($CD11c^{+}$ $CD123^{+/dim}$) and lymphoid ($CD123^{hi}$ $CD11c^{-}$).

There is a need to develop a protocol for generating BDC and in particular myeloid BDC and lymphoid BDC sub-populations. Such cells are useful as potential cellular agents for use, for example, in the manufacture of vaccines or in modulating immunological responsiveness. However, DC circulate in low number in the peripheral blood system and are, hence, difficult to isolate. A protocol is required, therefore, to generate a ready source of BDC.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The present invention provides a method for inducing or otherwise facilitating development of blood dendritic cells (BDC) from $CD34^+$ precursor cells. Generally, the $CD34^+$ precursor cells are from sources such as cord blood, bone marrow or peripheral blood. Cord blood is particularly preferred. The protocol generally involves sorting $CD34^+$ precursor cells into a myeloid population and/or a lymphoid population. The myeloid population is generally $CD33^+CD7^-CD10^-$ and the lymphoid population is generally $CD33^+CD7^+CD10^+$. One or both sub-populations of $CD34^+$ precursor cells are then cultured into the presence of one or more cytokines and preferably a cocktail of cytokines for a time and under conditions sufficient for $CD34^+$-derived DC to develop. The myeloid DC precursors differentiate *via* either CD14 or CD1a pathways. Within the expanded population, monocytes ($CD14^+$), granulocytes ($CD15^+$), myeloid BDC-like cells ($CD11c^+CD123^-$) and lymphoid BDC-like cells ($CD11c^-CD123^{hi}$) may develop. The latter myeloid and lymphoid BDC are proposed to be potential therapeutic cellular agents for the development of vaccines and to modulate immunological responsiveness.

The present invention provides, therefore, a method for generating myeloid- or lymphoid-like BDC, said method comprising isolating $CD34^+$ precursor cells, sorting into a myeloid and/or lymphoid population and culturing either or both populations in the presence of one or more cytokines or functional, recombinant or chemical homologs or equivalents thereof for a time and under conditions sufficient for $CD34^+$ cell expansion to occur and then isolating the $CD34^+$-derived myeloid- or lymphoid-like BDC.

The useful cytokines are, for example, flt3, SCF, IL-3, IL-6, GM-CSF, G-CSF and/or TNFα.

The ability to enrich for, or generate, myeloid- or lymphoid-like BDC permits a marked improvement over monocyte-derived DC. The isolated cells may be used to generate vaccines to induce an immunological response against specific antigens or may be used to induce immunological tolerance or non-responsiveness.

The present invention provides, therefore, an isolated population of lymphoid- or myeloid-like BDC for use in vaccine development or as potential therapeutic cellular agents to, for example, induce immunological tolerance or non-responsiveness.

The present invention further contemplates the use of myeloid- or lymphoid-like BDC derived from $CD34^+$ precursor cells in the manufacture of a population of potential therapeutic cellular agents.

The present invention also provides vaccines comprising the isolated myeloid- or lymphoid-like BDC loaded with particular antigens.

Reference to a "CD^-" includes a "CD^{lo}" or "$CD^{-/-}$" marker.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graphical representation showing the growth of cord blood (CB) $CD34^+$ myeloid precursors in the presence of flt3, SCF, IL-3 and IL-6 to generate an expanded culture.

Figure 2 is a graphical representation showing the emergence of $CD14^+$ progeny.

Figure 3 is a graphical representation showing the emergence of $CD15^+$ progeny.

Figure 4 is a graphical representation showing the emergence of $CD14^-$ $CD15^-$ progeny.

Figures 5(A)-(E) are graphical representations of $CD11c^+$ DC's existing in a $CD14^-$-$CD15^-$ population after precursor cell expansion.

Figure 6 is a graphical representation showing that $CD11c^+HLA\text{-}DR^+CD123^-CD1a^-$ cells can induce a potential mixed lymphocyte reaction (MLR).

DETAILED DESCRIPTION OF THE PREERRED EMBODIMENTS

The present invention provides a protocol for developing myeloid- or lymphoid-like BDC form $CD34^+$ precursor cells. Reference herein to "myeloid-like BDC" and "lymphoid-like BDC" includes myeloid BDC and lymphoid BDC, respectively.

Myeloid-like BDC have the potential for inducing more potent allogenic T-lymphocyte responses compared to granulocytes or monocytes and, hence, are proposed to be useful therapeutic cellular agents in the development of vaccines and to modulate immunological responsiveness.

Accordingly, one aspect of the present invention contemplates a method for generating a population of myeloid- or lymphoid-like BDC, said method comprising obtaining a population or source of $CD34^+$ precursor cells, sorting this population into myeloid and/or lymphoid precursors, culturing either or both populations with one or more cytokines for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC or lymphoid-like BDC from the expanded cell culture.

The $CD34^+$ precursor cells are preferably $CD34^+$ stem cells from any convenient source. The most convenient source is cord blood. Other sources include bone marrow and peripheral blood.

In relation to these preferred sources of $CD34^+$ precursor cells, another aspect of the present invention provides a method for generating a population of myeloid- or lymphoid-like BDC, said method comprising obtaining a population or source of $CD34^+$ stem cells from cord blood, bone marrow and/or peripheral blood sorting this population into myeloid and/or lymphoid precursors, culturing either or both populations with one or more cytokines for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC or lymphoid-like BDC from the expanded cell culture.

By "obtaining" $CD34^+$ precursor cells including stem cells means enriching, selecting and/or isolating $CD34^+$ cells from mixed populations of cells. The $CD34^+$ precursor cell culture does not have to be pure or solely or substantially $CD34^+$ cells but a substantially homologous population of $CD34^+$ cells is certainly preferred. The present invention extends, however, to heterogenous mixtures of cells provided the mixture comprises $CD34^+$ precursor-cells and in particular $CD34^+$ stem cells. Sorting of the $CD34^+$ precursor cells provides a population of myeloid precursors having characteristic markers $CD33^+CD7^-CD10^-$ and a population of $CD33^{+/-}CD7^+CD10^+$ lymphoid precursors. Either or both populations may then be subject to expansion-enhancing conditions. Although either myeloid or lymphoid precursor $CD34^+$ cells may be employed in the practice of the present invention, up to the present time, myeloid precursor cells are particularly preferred. Myeloid precursor $CD34^+$ cells give rise to myeloid-like BDC which are $CD11c^+CD123^-$ cells. Lymphoid precursor $CD34^+$ cells have the potential to give rise to lymphoid-like BDC which are $CD11c^-$ $CD123^{hi}$. Conveniently, $CD34^+$ cells may be collected by any convenient means such as being immobilized to magnetic particles or FACS sorting microspheres.

Sorting is preferably conducted with a flow cytometer which comprises a "fluorescence-activated cell sorter" (FACS) [see, for example, *"Methods in Cell Biology"* Vol. 33, Darzynkiewica, Z. and Crissman, H.A., eds., Academic Press) and Dangl and Herzenberg, *J. Immunol. Methods 52:* 1-14, 1982].

Accordingly, in a preferred embodiment, the present invention contemplates a method for generating a population of myeloid-like BDC, said method comprising obtaining a population or source of $CD34^+$ precursor cells, sorting this population to obtain myeloid precursor cells characterized by being $CD33^+CD7^-CD10^-$, culturing this population with one or more cytokines for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC characterized by being $CD11c^+CD123^-$ from the expanded cell culture.

Preferably, the $CD34^+$ precursor cells are derived or obtained from cord blood. However,

precursor cells from bone marrow and/or peripheral blood are also contemplated by the present invention.

The $CD34^+$ cell expansion conditions include incubating or culturing the cells in the presence of one or more cytokines. Preferably, a cocktail of two or more cytokines is employed. Although a range of cytokines may be employed, particularly useful cytokines are those selected from flt3, SCF, IL-3 and IL-6 or their functional, recombinant or chemical equivalents or homologs. Other useful cytokines include GM-CSF, G-CSF and TNFα.

Accordingly, another aspect of the present invention contemplates a method for generating a population of myeloid- or lymphoid-like BDC, said method comprising obtaining a population or source of $CD34^+$ precursor cells, sorting this population into myeloid and/or lymphoid precursors, culturing either or both populations with one or more cytokines selected from flt3, SCF, IL-3, IL-6, GM-CSF, G-CSF and TNFα or their functional, recombinant or chemical equivalents or homologs for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC or lymphoid-like BDC from the expanded cell culture.

Preferably, the $CD34^+$ precursor cells are $CD34^+$ cord blood-derived stem cells. Even more preferably, the cells generated are myeloid-like BDC characterized by being $CD11c^+CD123^-$.

A range of cytokines or functional equivalents may be employed and the present invention extends to any and all conditions which facilitate cell expansion. In a most preferred embodiment, however, a cocktail of cytokines comprising flt3, SCF, IL-3 and IL-6 is employed.

Accordingly, another aspect of the present invention contemplates a method for generating a population of myeloid- or lymphoid-like BDC, said method comprising obtaining a population or source of $CD34^+$ precursor cells, sorting this population into myeloid and/or

lymphoid precursors, culturing either or both populations with a mixture of cytokines comprising flt3, SCF, IL-3 and IL-6 or their functional, recombinant or chemical equivalents or homologs of any or all of the above for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC or lymphoid-like BDC from the expanded cell culture.

In another preferred embodiment, the present invention provides a method for generating a population of myeloid- or lymphoid-like BDC, said method comprising obtaining a population or source of $CD34^+$ stem cells from cord blood, bone marrow and/or peripheral blood, sorting this population into myeloid and/or lymphoid precursors, culturing either or both populations with a mixture of cytokines comprising flt3, SCF, IL-3 and IL-6 and optionally one or more of GM-CSF, G-CSF and TNFα or their functional, recombinant or chemical equivalents or homologs of any or all of the above for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC or lymphoid-like BDC from the expanded cell culture.

In yet another embodiment, the present invention contemplates a method for generating a population of myeloid-like BDC, said method comprising obtaining a population or source of $CD34^+$ precursor cells, sorting this population to obtain myeloid precursor cells characterized by being $CD33^+CD7^-CD10^-$, culturing both populations with a mixture of cytokines comprising flt3, SCF, IL-3 and IL-6 and optionally one or more of GM-CSF, G-CSF and TNFα or their functional, recombinant or chemical equivalents or homologs of any or all of the above for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC characterized by being $CD11c^+CD123^-$ from the expanded cell culture.

The myeloid- and lymphoid-like BDC populations obtainable by the method of the present invention are proposed to be useful in the generation of vaccines and to modulate immunological responsiveness.

The present invention provides, therefore, a population of cells comprising myeloid- or

lymphoid-like BDC, said population of cells isolated or enriched from an expanded culture of $CD34^+$ cells generated from myeloid or lymphoid precursor cells sorted from a population of $CD34^+$ precursor cells and cultured in the presence of one or more cytokines.

Reference to a "population" includes reference to an isolated culture comprising a homogenous, a substantially homogenous or a heterogenous culture of cells. Preferably, the population of myeloid-or lymphoid-like cells is substantially homogenous for one or either of these types of cells. Generally, a "popluation" may also be regarded as an "isolated" culture of cells.

In a preferred embodiment, a cocktail of cytokines is used comprising two or more of flt3, SCF, IL-3 and/or IL-6 or functional, recombinant or chemical equivalents thereof. Even more preferably, a cocktail comprises at least all of the flt3 ligand, SCF, IL-3 and IL-6.

In a particularly preferred embodiment, the present invention further extends to a population of myeloid-like BDCs, said population generated by the method of obtaining a population or source of $CD34^+$ precursor cells, sorting this population into myeloid and/or lymphoid precursors, culturing either or both populations with one or more cytokines selected from flt3, SCF, IL-3 and IL-6 and optionally one or more of GM-CSF, G-CSF n TNFα or their functional, recombinant or chemical equivalents or homologs for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC or lymphoid-like BDC from the expanded cell culture.

The isolated, population of myeloid- and/or lymphoid-like cells are useful in the manufacture of vaccines. In one embodiment, the cells are exposed, incubated or cultured with one or more antigens for a time and under conditions for the antigen to be taken up by the cells. Upon re-introduction of the antigen-load myeloid- or lymphoid-like BDC, an immune response is generated against the antigens. These antigens may be derived from pathogenic, microorganisms, parasites, cancer cells or other sources.

Vaccines are also useful in the treatment or prophylaxis of inflammatory bowel disease and

to increase levels of immune responsiveness such as during stress (e.g. surgery) or chemotherapy.

Alternatively, the myeloid- or lymphoid-like BDC may be loaded with sub-optimal levels of antigen or loaded with a super dose which can result in the induction of immuno-tolerance or immuno-non-responsiveness.

Accordingly, another aspect of the present invention contemplates a method of vaccinating a subject against an antigen including a cell carrying the antigen, said method comprising loading a myeloid- or lymphoid-like BDC with an amount of said antigen which will induce an immune response wherein said myeloid- or lymphoid-like BDC or its parent is prepared by the method of generating a population of lymphoid- or myeloid-like BDC, said method comprising obtaining a population or source of $CD34^+$ precursor cells, sorting this population into myeloid and/or lymphoid precursors, culturing either or both populations with one or more cytokines for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture and then isolating myeloid-like BDC or lymphoid-like BDC from the expanded cell culture.

Preferably, the $CD34^+$ precursor cells are $CD34^+$ stem cells from cord blood.

Preferably, the myeloid and/or lymphoid precursor cells are cultured in the presence of a cytokine selected from the list comprising flt3, SCF, IL-3 and IL-6. Even more preferably, the cells are cultured in a cocktail of cytokines comprising flt3-ligand, SCF, IL-3 and IL-6. Other cytokines in the cocktail may optionally include GM-CSF, G-CSF and TNFα.

In a most preferred embodiment, the present invention contemplates a method of vaccinating a subject against an antigen including a cell carrying the antigen, said method comprising loading a myeloid-like BDC with an amount of said antigen which will induce an immune response wherein said myeloid-like BDC or its parent is prepared by the method of generating a population of myeloid-like BDC by obtaining a population or source of $CD34^+$ precursor cells, sorting this population into myeloid precursors, culturing

this population with one or more cytokines for a time and under conditions sufficient to obtain a $CD34^+$-derived cell expansion culture, loading the myeloid-like BDC with an antigen and then introducing the BDC to the subject. Generally, the subject is the source of the $CD34^+$ precursor cells.

In another embodiment, as indicated above, the myeloid- or lymphoid-like BDC are loaded with sub-optimal or excess doses of antigen to induce immuno-tolerance or non-responsiveness.

The present invention provides, therefore, compositions for modulating the immune system or a response thereby, said composition comprising a myeloid- or lymphoid-like BDC generated by the method as hereinbefore described or are progeny cells of these generated cells. Such cells may be optionally loaded with antigen to induce a protective immune response or with sub- or over-optimum levels to induce immuno-tolerance or non-responsiveness.

Generally, although not exclusively, when used in therapy, autologous BDC are used relative to the subject.

The preferred subject is a human but the present invention extends to other primates, livestock animals (e.g. sheep, cows, horses, pigs, donkeys, goats), companion animals (e.g. dogs, cats) or laboratory test animals (e.g. mice, rabbits, guinea pigs, hamsters) and avian species (e.g. chickens, game birds or aviary birds).

The present invention further contemplates the use of myeloid- or lymphoid-like BDC generated as hereinbefore described in the manufacture of a vaccine or therapeutic cellular agent.

Such a vaccine or therapeutic cellular agent is also regarded herein as a medicament.

In one embodiment, the vaccine or therapeutic cellular agent is useful for the treatment or

prophylaxis of cancer infection by pathogenic microorganism, parasite or virus or to induce immuno-tolerance or non-responsiveness such as in the treatment or prophylaxis of autoimmune disease conditions.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Generation of myeloid-like BDC

Sorted myeloid ($CD33^+CD7^-CD10^-$) and lymphoid ($CD34^+CD33^{+/-}CD7^+CD10^+$) precursors from enriched cord blood $CD34^+$ cells were cultured in 24-well plates (4-5 $x10^4$ cells/ml) in H2000 serum free medium supplemented with a cocktail of cytokines (flt3-ligand 50 ng/ml, SCF 50 ng/ml, IL-3 10 ng/ml and IL-6 10 ng/ml) for 2-3 weeks. Figure 1 shows the growth of cord blood $CD34^+$ precursors in the presence of the cytokines. The progeny were assessed for phenotype on days 6-8 and every second day thereafter and also for their capacity to induce allogeneic T lymphocyte responses on days 8-12, of culture.

The presence of $CD11c^+$ myeloid-like BDC in a $CD14^-CD15^-$ population is shown in Figures 5A-E.

Figures 2-4 show the emergence of $CD14^+$, $CD15^+$ and $CD14^-CD15^-$ populations and Figure 5 shows the emergency of $CD11c^+CD14^-CD15^-$ progeny.

Figure 6 shows $CD11c^+HLA\text{-}DR^+CD123^-CD1a^-$ cells can induce a mixed lymphocyte reaction (MLR).

Peak $CD34^+$ derived cell expansion was observed on day 10-13 of culture, resulting in a 70 to 100 fold increase in cell number. Thereafter, cell number decreased steadily, although viable cells (>60%) could be observed for up to 3 weeks. There was no evidence of lineage differentiation during the initial period (4-6 days) of cell expansion. Monocytes ($CD14^+$ cells), granulocytes ($CD15^+$ cells), and myeloid-like BDC ($CD11c^+CD123^-$ cells) appeared subsequently (days 6-8) and were maintained during the period of culture. Only the $CD11c^+CD123^-$ progeny were capable of inducing potent allogeneic T lymphocyte responses, compare to monocytes and granulocytes.

EXAMPLE 2

Yield of CD34$^+$, myeloid/lymphoid precursors

The yield of CD34$^+$, myeloid/lymphoid precursor is shown in Table 1.

TABLE 1

Sample	Volume	MNC	CD34$^+$	Myeloid precursor	Lymphoid precursor
CB 20	50 ml	350×10^6	5.8×10^6	0.6×10^6	0.12×10^6
CB 53	50 ml	300×10^6	4×10^6	0.5×10^6	0.02×10^6
CB 56	55 ml	310×10^6	3.5×10^6	0.3×10^6	0.04×10^6
CB 30*	-	360×10^6	$1.8\text{-}2.5 \times 10^6$	-	-

Purity CD34$^+$ cells: >98%

* Pranke, *Acta Haematologica 105:* 71-76, 2001

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

BIBLIOGRAPHY

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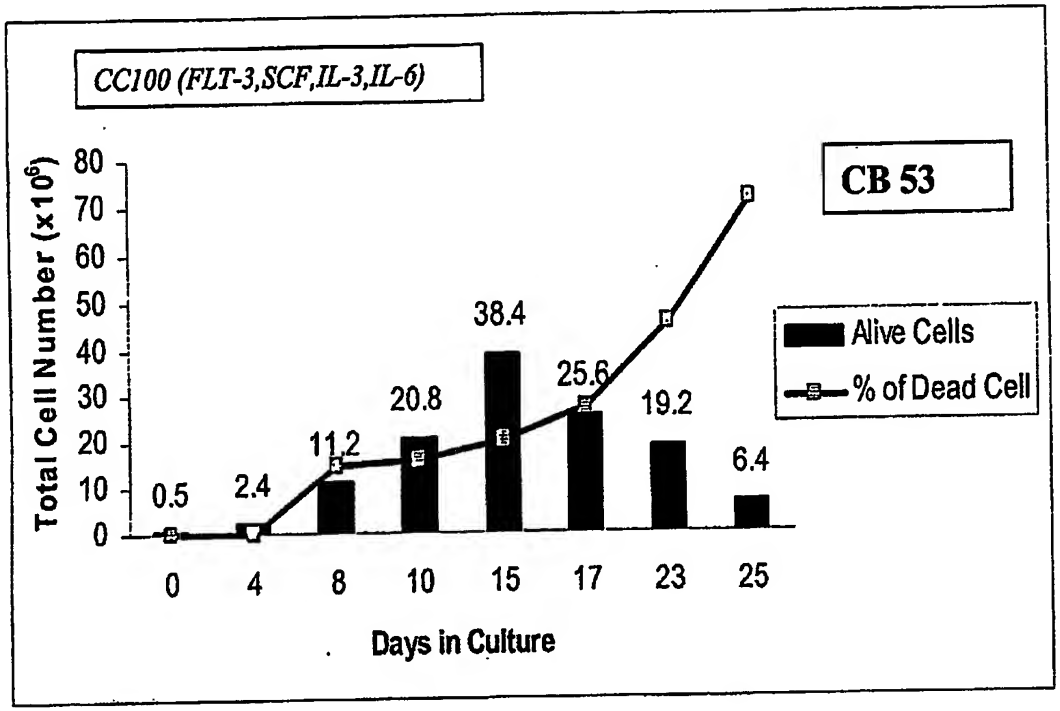
Grouard *et al., J. Exp. Med. 185:* 1101-1111, 1997.

Hart, *Blood 90:* 3245-3287, 1997.

Pranke, *Acta Haematologica 105:* 71-76, 2001

DATED this thirtieth day of August 2002.

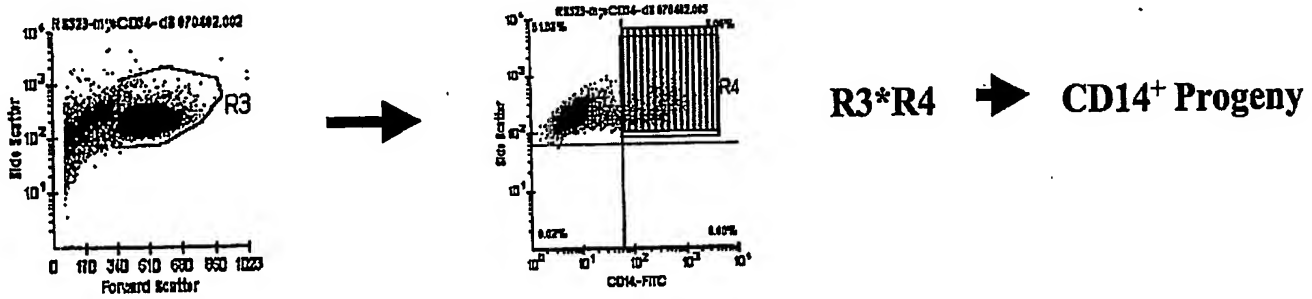
The Corporation of the Trustees of the Order of the Sisters of Mercy in Queensland
by DAVIES COLLISION CAVE
Patent Attorneys for the Applicant



*All Data derived from myeloid precursor

Similar results from 4 exp.

Figure 1


R3
R4
Side Scatter
Forward Scatter
CD14-FITC
R3*R4
CD14+ Progeny

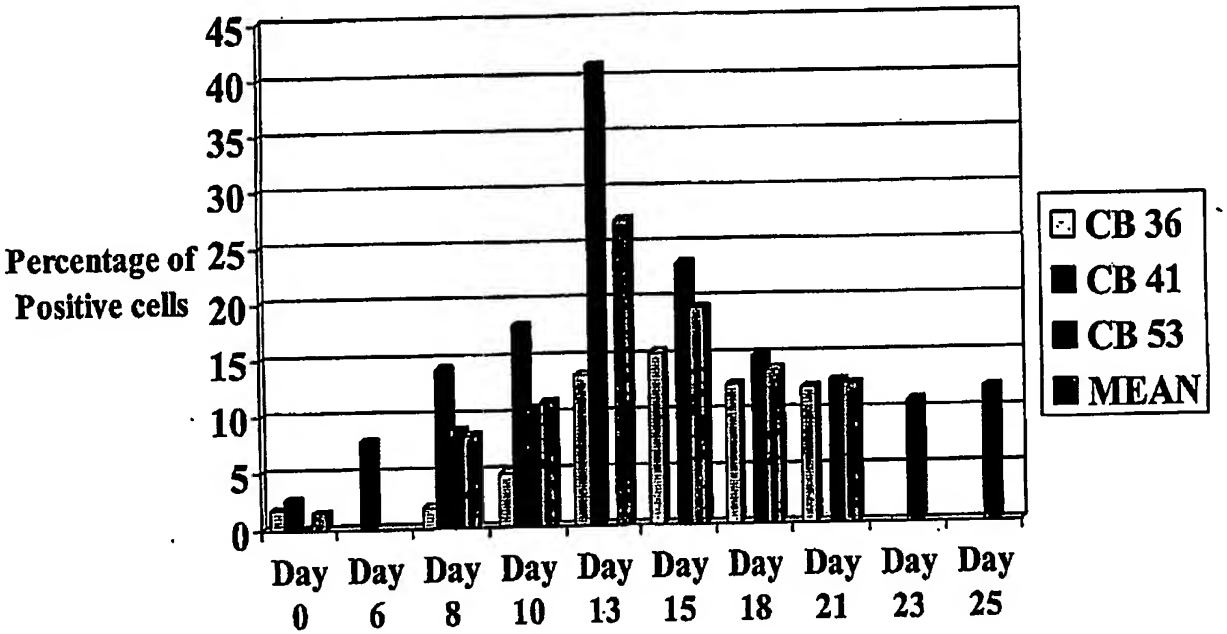

Percentage of Positive cells
45
40
35
30
25
20
15
10
5
0
Day 0
Day 6
Day 8
Day 10
Day 13
Day 15
Day 18
Day 21
Day 23
Day 25
CB 36
CB 41
CB 53
MEAN

Figure 2

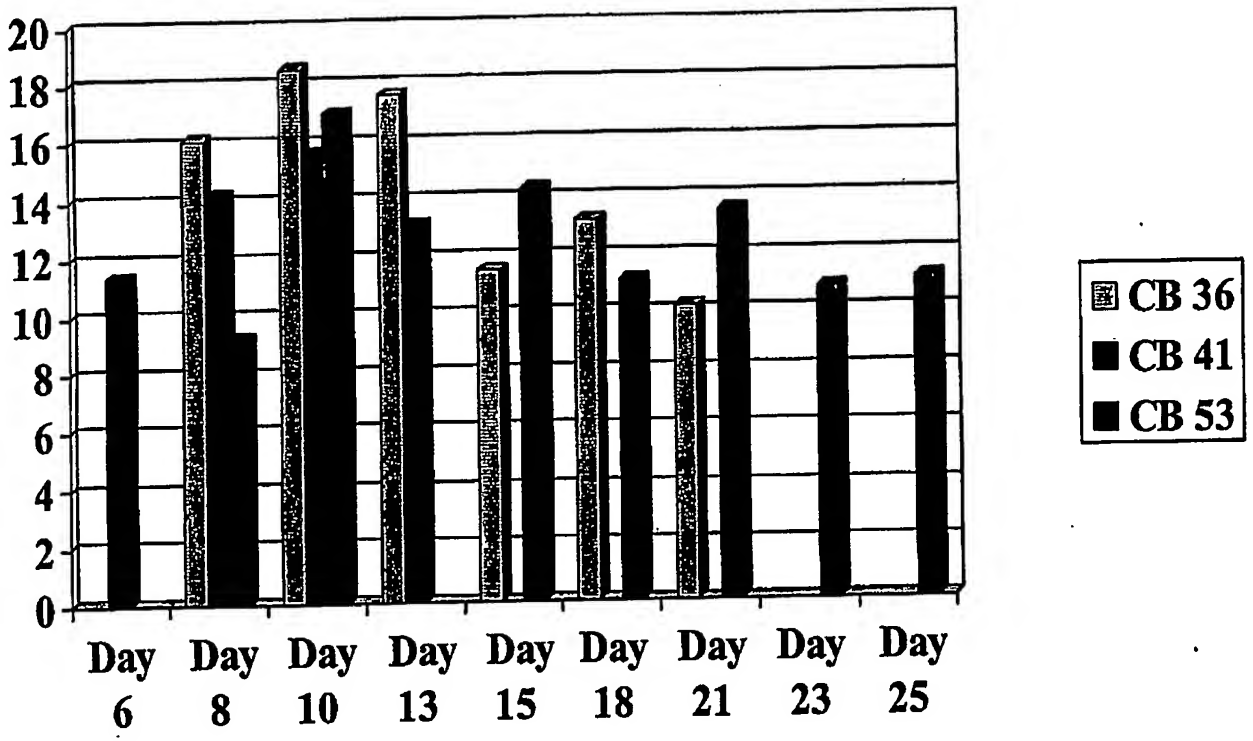
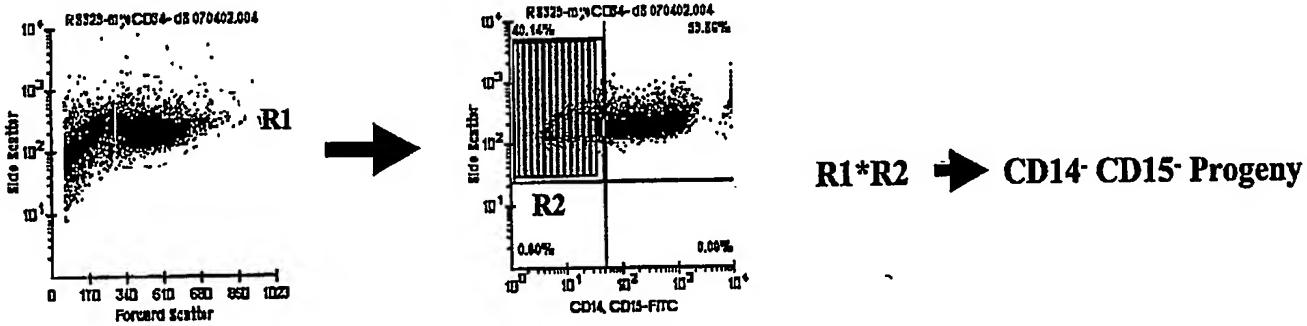

20
18
16
14
12
10
8
6
4
2
0
Day 6
Day 8
Day 10
Day 13
Day 15
Day 18
Day 21
Day 23
Day 25
CB 36
CB 41
CB 53

Figure 3


R1
R2
Side Scatter
Forward Scatter
CD14, CD15-FITC
R1*R2
CD14- CD15- Progeny

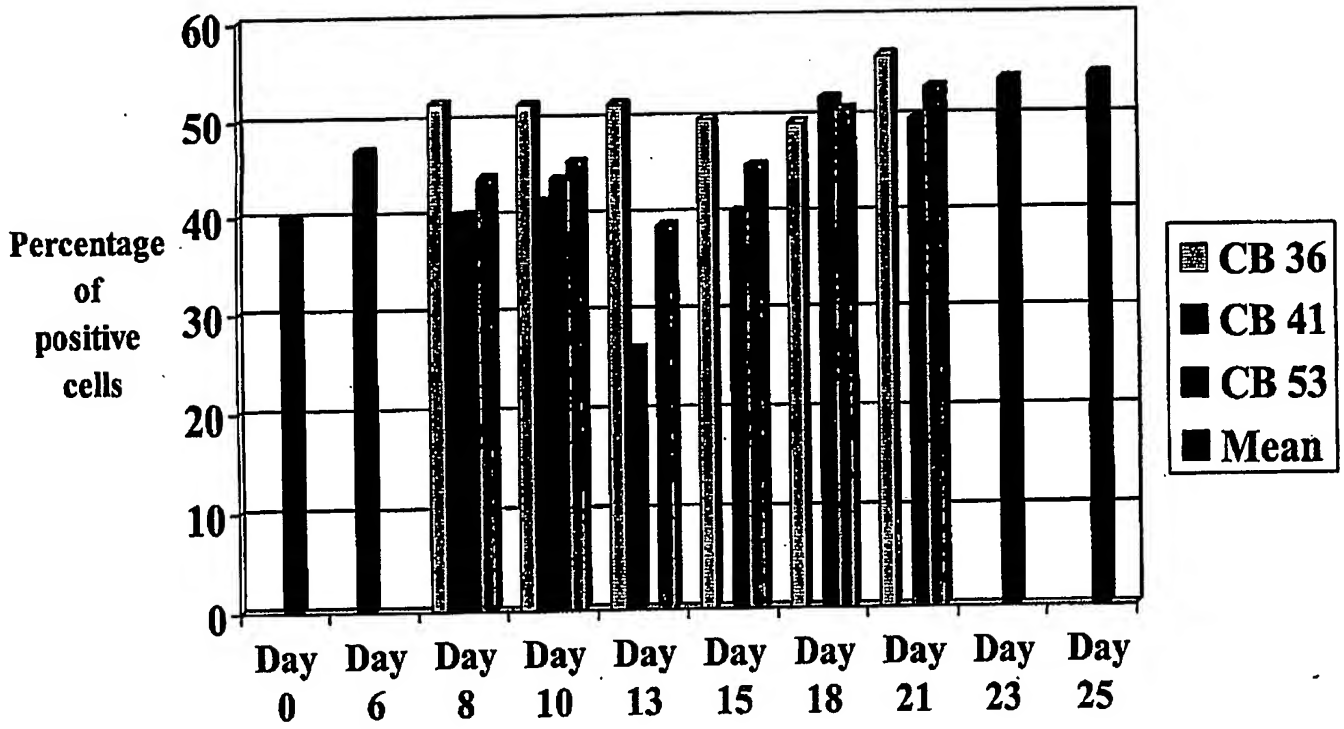

Percentage of positive cells
60
50
40
30
20
10
0
Day 0
Day 6
Day 8
Day 10
Day 13
Day 15
Day 18
Day 21
Day 23
Day 25
CB 36
CB 41
CB 53
Mean

Figure 4

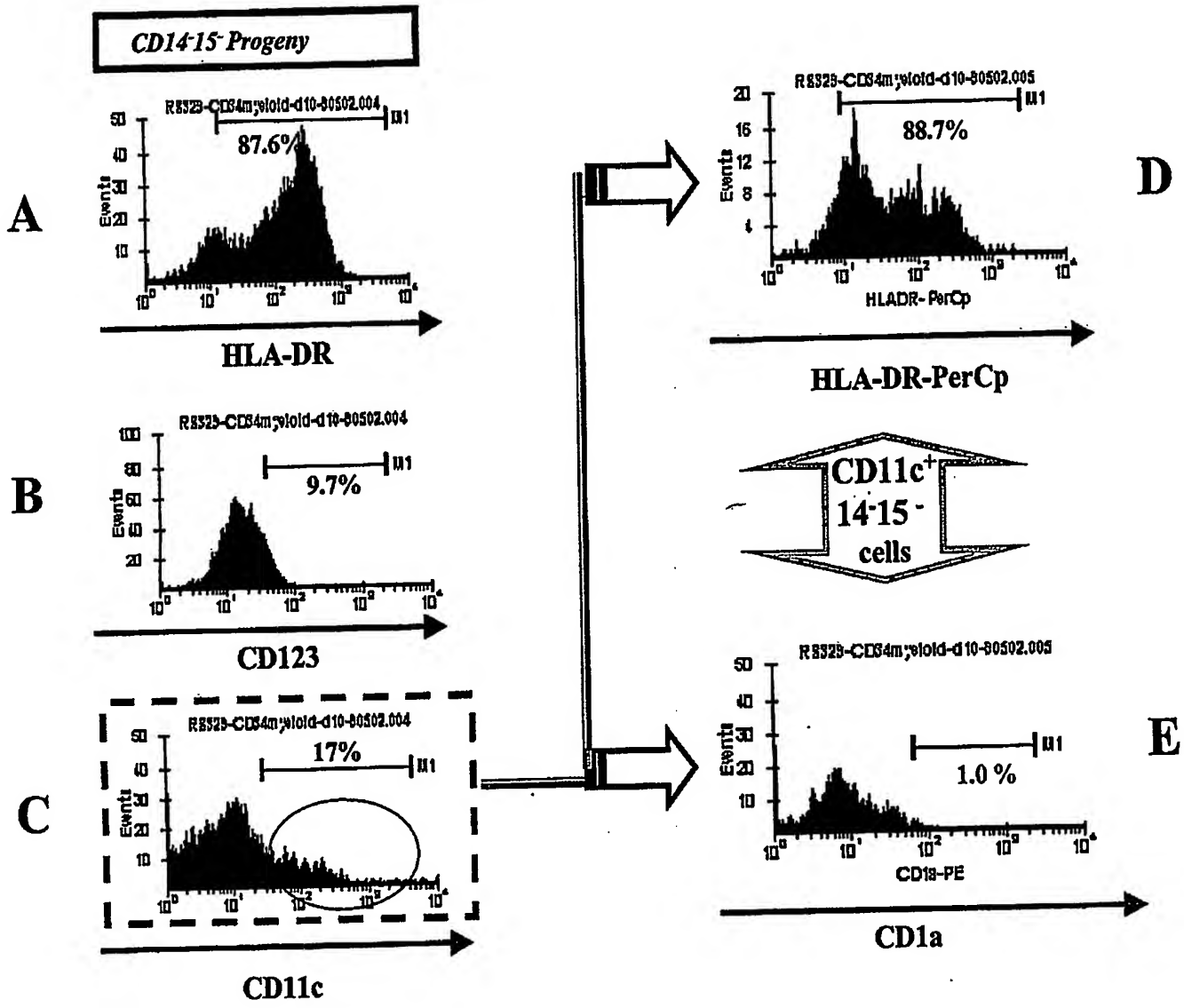

CD14-15- Progeny
A
87.6%
HLA-DR
B
9.7%
CD123
C
17%
CD11c
D
88.7%
HLADR-PerCp
HLA-DR-PerCp
CD11c+ 14-15- cells
E
1.0 %
CD1a

Figure 5

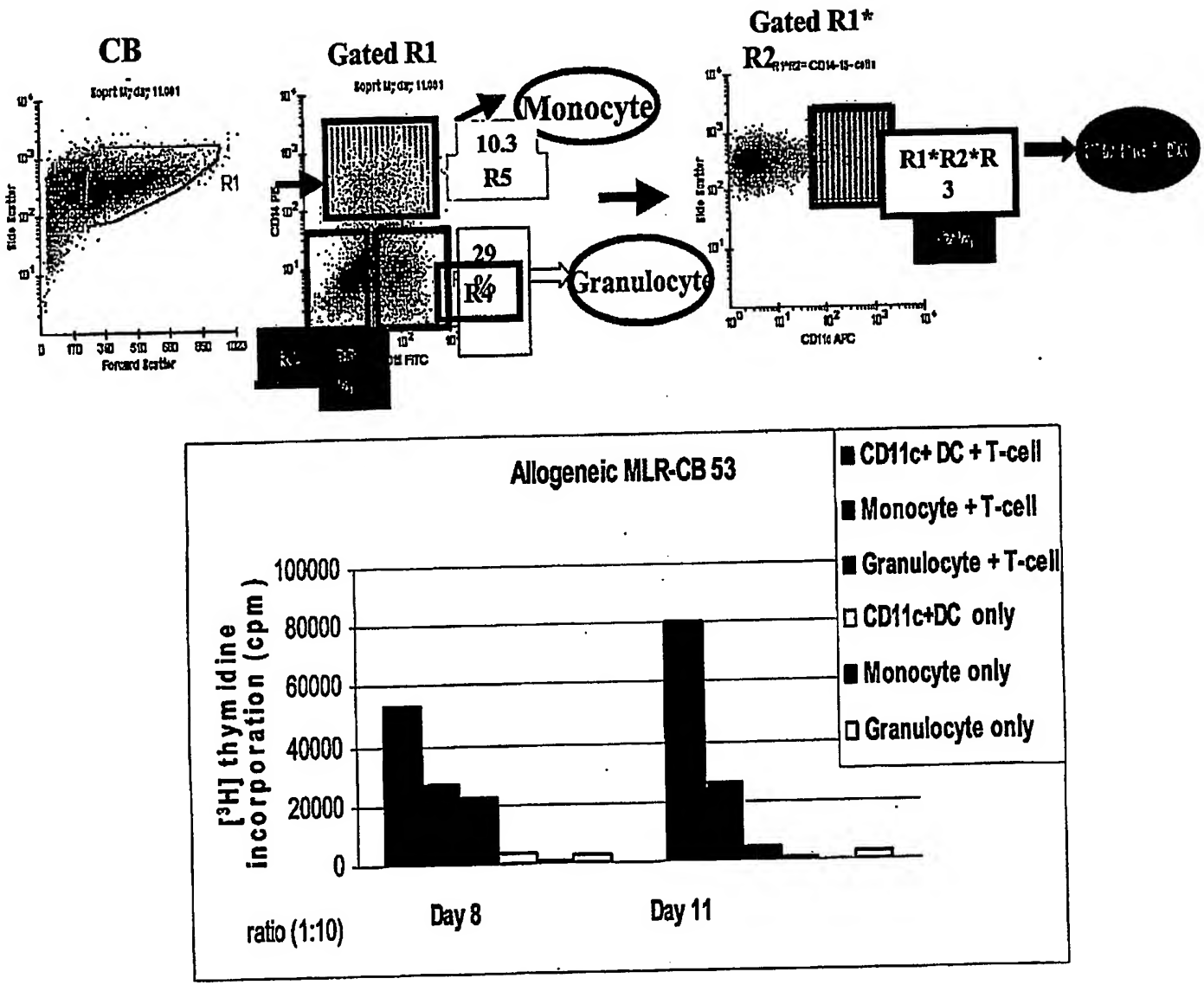

CB
Gated R1
Gated R1*
R2
R1
Monocyte
10.3
R5
29
R4
Granulocyte
R1*R2*R
3
CD11c APC
Allogeneic MLR-CB 53
CD11c+ DC + T-cell
Monocyte + T-cell
Granulocyte + T-cell
CD11c+DC only
Monocyte only
Granulocyte only
[3H] thymidine incorporation (cpm)
100000
80000
60000
40000
20000
0
Day 8
Day 11
ratio (1:10)

Figure 6